TEMPERATURE EFFECT ON A CELL FREE PROTEIN SYNTHESIS OF RAT TESTIS

Masahisa NAKAMURA, Peter F. HALL* and Junzo KATO

Department of Obstetrics and Gynecology, Teikyo University, 2-11-11 Kaga, Itabashi-ku, Tokyo 173, Japan and **Department of Physiology, University of California, Irvine, CA 92717, USA*

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1. Introduction

According to many histological studies, transplantation of the testis from scrotal sac to the abdominal cavity (artificial cryptorchidism) is known to result in disappearance of the differentiating germinal epithelial cells from the seminiferous tubules [1–3], whereas spermatogonia, Sertoli cells and Leydig cells seem to be relatively unaffected [4,5]. The more mature germinal cells of the testis appear to be the most susceptible to temperature elevation [4].

Incorporation of labeled amino acid into protein of slices of cryptorchid rat testis was greater at 37°C than at 34°C in contrast to declined protein labeling in scrotal testis at 37°C [6]. However, the mechanism by which a small elevation of temperature causes the decline of protein synthesis by scrotal testis and the elevation of protein synthesis by cryptorchid testis, is poorly understood, although many studies have been done to examine the mechanism by which body temperature affects protein synthesis in the rat testis [3,7,10]. Pactamycin is known to inhibit an initiation phase of protein synthesis [8]. Here we report by use of pactamycin, the influence of temperature elevation on protein synthesis in a cell free system from rat testis.

2. Materials and methods

2.1. *Animals*

Sprague-Dawley rats were used for the experiments. Thirty days before sacrifice, rats were made cryptorchid on one side by the method in [8]. After sacrificing animals, testes were removed quickly, placed on ice, decapsulated and weighed. Portions of testis were gently teased off with fine forceps and the rest of the testis was lysed as in [9]. Lysate was centrifuged at 30 000 × *g* for 20 min at 1°C. The resultant supernatant was loaded on Sephadex G-25 column (1.6 × 22 cm) equilibrated with a buffer as given in [9]. The first 2.5 ml effluent from void volume was collected and used for experiments.

2.2. *[^{3}H]Amino acid incorporation into protein*

Lysate was incubated for various times at 34°C and 37°C with [^{3}H]phenylalanine or [^{3}H]leucine and other necessary components [10]. Small portions of testis were also incubated for 60 min at 34°C and 37°C in 3 ml of phosphate-buffered saline containing 10 mM glucose (pH 7.4) with shaking at 80 osc./min. Incorporation of [^{3}H]amino acid into protein was terminated by addition of 3 ml of 10% trichloroacetic acid (TCA), then tissue homogenized with a glass–glass homogenizer. Hot TCA-insoluble materials were filtrated onto Millipore filter membrane and [^{3}H]-amino acid into protein was measured by the method in [10]. Protein was determined by the method in [11] with bovine serum albumin as standard.

2.3. *Preparation of round spermatids*

Round spermatids (stage 1–8) were isolated by the method in [10]. Lysate of spermatids was prepared and incubated with [^{3}H]phenylalanine and other necessary components with or without pactamycin as in [7,10].

2.4. *Preparation of 'ribosomes' and high speed supernatant*

Lysate from whole tissue was further fractionated by centrifugation at 180 000 × *g* for 3 h at 1°C. The resultant precipitation and the supernatant are designated as the ribosome fraction and the high speed supernatant, respectively. Then ribosomes were

resuspended as in [12]. The 2 components, ribosomes and high-speed supernatant were incubated at 34°C and 37°C for 5 min separately [12] and the 2 were mixed prior to measurement of protein at 34°C for 60 min. Incorporation of [^{3}H]phenylalanine into protein was measured as mentioned above.

Pactamycin was a kind gift of Upjohn Co. (Kalamazoo, MI) and other sources of various chemicals have been reported in [10].

2.5. *Preparation for microscopy*

Intact scrotal and cryptorchid testes were fixed and prepared for both light and electron microscopy as in [14].

3. Results

Cryptorchid rat testis was examined under electron microscopy and revealed to consist of spermatogonia, Sertoli cells and Leydig cells as in [13]. For 30 days following fixation of the abdominal transplantation of the adult testis on the right, the weight of testis decreased remarkably (0.300 ± 0.016 g; mean ± SE from 5 animals), compared to that of the scrotal testis (1.407 ± 0.068 g). Table 1 indicates the incorporation of [^{3}H]phenylalanine into protein of whole tissue or lysate from cryptorchid and scrotal testis in vitro at 34°C and 37°C. Incorporation of [^{3}H]amino acid into protein of cryptorchid testis was greater at 37°C than at 34°C in both whole tissue and lysate, whereas scrotal testis incorporated less [^{3}H]phenylalanine into protein at 37°C than at 34°C. The results from whole tissue are in agreement with [6,15]. In a cell free system, incorporation of [^{3}H]amino acid into protein of cryptorchid testis was also greater at 37°C than at 34°C. On the contrary, lysate of scrotal testis incorporated less [^{3}H]amino acid into protein at 34°C. Fig.1 shows the time course for incorporation

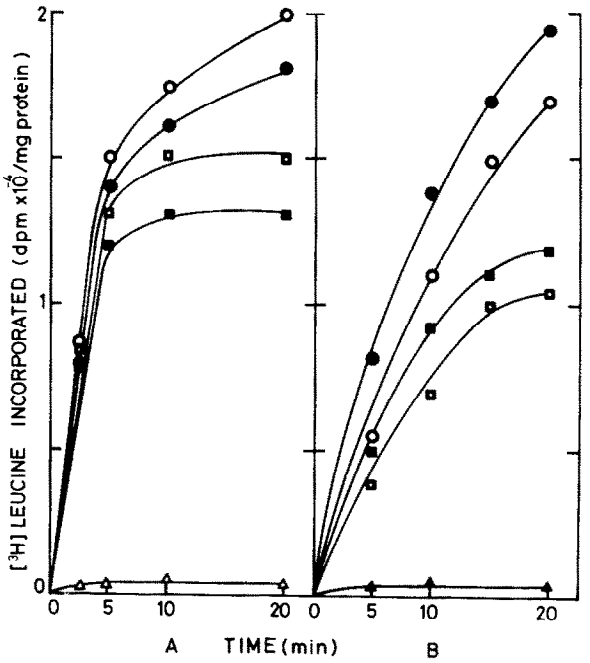

Fig.1. [^{3}H]Leucine incorporation into protein by a lysate from scrotal and cryptorchid rat testis. Lysates were incubated at 34°C and 37°C for various times shown with 5 μCi [^{3}H]leucine (0.09 nmol/flask). Incubation was terminated by addition of equal volume of 10% TCA and [^{3}H]leucine incorporation was measured by TCA precipitation [10]. (A) Scrotal testis. Cryptorchid testis. Open symbols, 34°C; closed symbols, 37°C; circles, no addition; squares, pactamycin (5×10^{-6} M); triangles, pactamycin (5×10^{-6} M) + cycloheximide (3 mM).

Table 1
[^{3}H]phenylalanine incorporation into protein of rat testis

	Testis	[^{3}H]phenylalanine incorporated (dpm × 10^{-4}/mg protein)	
		34°C	37°C
Whole tissue	Scrotal	3.99 ± 0.21 (3)	3.30 ± 0.21
	Cryptorchid	7.16 ± 0.49 (3)	8.80 ± 0.26
Lysate	Scrotal	3.64	3.22
	Cryptorchid	2.37	2.97

Whole tissue and lysate were incubated with [^{3}H]phenylalanine (5 μCi; 0.1 nmol/flask). Number of experiments is shown in parenthesis. Values in whole tissue are the means ± SE

of [^{3}H]leucine into protein in a lysate from cryptorchid and scrotal testis in the presence or absence of pactamycin (5×10^{-6} M) with or without cycloheximide (3 mM). As shown in fig.1A, more [^{3}H]leucine was incorporated into protein of lysate from scrotal testis at 34°C than at 37°C at all the times examined. It was also true that lysate from cryptorchid testis was labeled more with [^{3}H]amino acid at 37°C than at 34°C (fig.1B). In the presence of pactamycin, protein synthesis was greater at 37°C in cryptorchid testis than at 34°C, whereas it was less at 37°C in scrotal testis than at 34°C.

As shown in fig.2, it was found that in a lysate of spermatids labeling of protein with [^{3}H]phenylalanine was greater at 34°C than at 37°C as in [7] and that in the presence of pactamycin [^{3}H]amino acid incorporation was greater at 34°C than at 37°C. Cycloheximide (3 mM) completely inhibited protein synthesis (fig.1,2).

When lysate from whole tissue was further fractionated, and the resultant precipitation, designated as ribosomes and high speed supernatant preincubated for 5 min at 34°C and at 37°C separately, and the reconstituted system incubated for 60 min at 34°C, the activity of ribosomes and high speed supernatant was examined. It is shown in table 2 that the activity of ribosomes from scrotal testis decreased by a short time exposure to 37°C without any detectable change in the activity of the high speed supernatant. On the other hand, the activity of ribosomes and high speed supernatant from cryptorchid testis remained unchanged under the same condition.

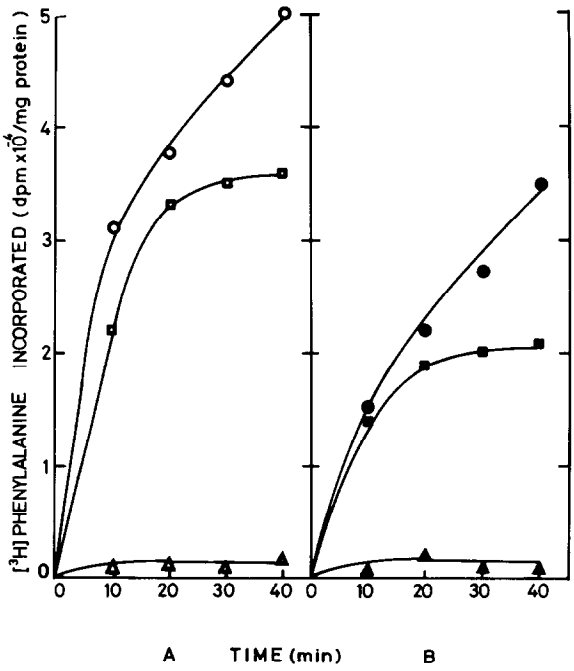

Fig.2. [^{3}H]phenylalanine incorporation into protein by a lysate from round spermatids. Lysate was incubated with 5 μCi [^{3}H]phenylalanine (0.1 nmol/flask) as in section 2. (A) 34°C. (B) 37°C. Circles, no addition; squares, pactamycin (5×10^{-6} M); triangles, pactamycin (5×10^{-6} M) + cycloheximide (3 mM).

Table 2
Effect of temperature on ribosomes and high speed supernatant in a cell free protein synthesis of scrotal and cryptorchid testis

	Prewarming (°C)		[^{3}H]phenylalanine incorporated (dpm × 10^{-4}/mg protein)
	Supernatant	Ribosomes	
Scrotal testis	34	34	1.73 ± 0.07
	34	37	1.33 ± 0.07
	37	34	1.71 ± 0.04
	37	37	1.27 ± 0.02
Cryptorchid testis	34	34	1.84 ± 0.07
	34	37	1.89 ± 0.11
	37	34	1.85 ± 0.20
	37	37	1.84 ± 0.11

Incubation was performed as in section 2. Values are the means ± SE

4. Discussion

This study has clearly demonstrated that the inhibition of body temperature on protein synthesis of scrotal testis and the increase on that of cryptorchid testis can be seen in a cell free system as well as in whole tissue. It is possible therefore that influence of temperature on protein synthesis is partly explained by an inhibition or a stimulation for some steps in incorporation of amino acids into protein, or by change for some steps in initiation, elongation and termination of peptide chains, apart from an effect of body temperature on transport of substrates like amino acids into cells. Since pactamycin (5×10^{-6} M) and cycloheximide (3 mM) are reported to block chain initiation and chain elongation, respectively [8], the difference between values at plateau with pactamycin and with pactamycin and cycloheximide (fig.1,2), is equivalent to the radioactivity associated with one round of translation. It is thus reasonable to presume that in scrotal and cryptorchid testis the change in protein synthesis by temperature elevation is at least due to the change in one round translation of protein synthesis (fig.1,2). As shown in fig.2A,B, the decreased amount of one round translation in round spermatids at 37°C may in part contribute to the decline in protein synthesis of scrotal testis, since it is reported that in scrotal testis, spermatids are in the high population [16] and seem to be one of the most sensitive cells to temperature elevation among various types of testicular cells [3]. This is supported by the fact that a cell free protein synthesis of spermatids was observed much more sensitive to temperature elevation than that of spermatocytes (data not shown).

It has been also reported that the elevation of protein synthesis of cryptorchid testis at body temperature is brought by the increase in protein synthesis of Sertoli cells [13]. The elevation of protein synthesis in a lysate from cryptorchid testis shown in fig.1B might therefore result from Sertoli cells, or from an increase of one round translation of protein synthesis. However, it is not possible to exclude the possibility that spermatogonia and/or Leydig cells might cause the elevation of protein synthesis in a lysate of cryptorchid testis as seen in fig.1B.

The results in table 2 also suggest that one round translation of protein synthesis decreased by temperature elevation in scrotal testis, might be partly due to the loss of activity of ribosomes. On the other hand, ribosomes from cryptorchid testis were relatively resistant to body temperature (table 2). This resistance of ribosomes in cryptorchid testis might be one of the reasons why Sertoli cells, Leydig cells and spermatogonia are relatively more resistant to heat than the differentiating germinal cells. However, it still remains unclear how body temperature changes one round translation of peptide chains and impairs the ribosomes. Further studies will answer this question.

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References

[1] Fukui, N. (1923) Acta Sch. Med. Univ. Kioto 6, 226–259.
[2] Moore, C. R. (1923) Anat. Rec. 24, 383.
[3] VanDemark, N. L. and Free, J. J. (1970) in: The Testis, (Johnson, A. D. et al. eds) vol. 3, pp. 233–312. Academic Press, New York.
[4] Nelson, W. O. (1951) Rec. Prog. Horm. Res. 6, 29–62.
[5] Williams, W. L. and Cunningham, B. (1940) Yale J. Biol. Med. 12, 309–316.
[6] Davis, J. R., Firlit, C. F. and Hollinger, M. A. (1963) Am. J. Physiol. 204, 696–698.
[7] Nakamura, M. and Hall, P. F. (1978) Biochem. Biophys. Res. Commun. 85, 756–761.
[8] Vazquez, D. (1974) FEBS Lett. 40, S63–S84.
[9] Lodish, H. F. (1974) Methods Enzymol. 709–723.
[10] Nakamura, M. and Hall, P. F. (1978) J. Cell Biol. 79, 1–9.
[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[12] Mizuno, S. (1975) Biochim. Biophys. Acta 414, 273–282.
[13] Firlit, C. F. and Davis, J. R. (1966) J. Reprod. Fert. 11, 126–131.
[14] Romrell, L. J., Bellvé, A. R. and Fawcett, D. W. (1976) Dev. Biol. 49, 119–131.
[15] Davis, J. R., Bergh, A. and Janson, P. O. (1965) J. Reprod. Fert. 10, 149–152.
[16] Meistrich, M. L., Bruce, W. R. and Clermont, Y. (1973) Exptl. Cell Res. 79, 213–227.